Original Article

Application of UV spectrophotometric method to study stress degradation behavior of cefprozil

Abstract

Background: Cefprozil is a bactericidal drug that is used in the treatment of susceptible infections including upper and lower respiratory tract infections and skin and soft tissue infections. **Materials and Methods:** The objective of this research work was to develop and validate new, simple ultraviolet (UV) spectrophotometric method of Cefprozil in bulk and pharmaceutical dosage form and its application to study its stress degradation behavior. **Results:** The absorbance maxima peak was found at 280 nm, and linearity was observed in the concentration range of 2-10 μg/ml. The method was validated and found to be precise. Accuracy (percent recovery) for Cefprozil was found to be 99.117±1.005. **Conclusion:** A new method for estimation of Cefprozil by UV spectrophometry was developed and validated and Cefprozil was found to undergo degradation in all stress conditions.

Key words:
Cefprozil, stress degradation, UV spectroscopy, validation

Introduction

Chemically, Cefprozil is (6R, 7R)-7-[(R)-2-Amino-2-(p-hydro xyphenyl) acetamido]-8-oxo-3-propenyl-5-thia-1-azabicyclo [4.2.0] oct-2-ene-2-carboxylic acid monohydrate [Figure 1]. Cefprozil is a bactericidal drug used in the treatment of susceptible infections including upper and lower respiratory tract infections and skin and soft tissue infections and should probably be classified as a second-generation cephalosporin's, beta-lactum, and other inhibitors of cell-wall synthesis.[1-3]

Spectrophotometry is generally preferred, especially by small-scale industries as the cost of the equipment is less and the maintenance problems are minimal. The method of analysis is based on measuring the absorption of a monochromatic light by colorless compounds in the near ultraviolet (UV) path of spectrum (200-380 nm). UV spectrophotometry can be used for stress-degradation studies of Cefprozil and its degraded products. The active pharmaceutical ingredient is subjected to a number of forced degradation conditions to include acidic, basic, and oxidative conditions as per ICH guidelines.[4] Forced degradation should be one of the activities performed early in the development process to ensure that the method is discriminating before a lot of time, effort, and money have been expended. It is important to determine the conditions responsible to degrade the drug.

Earlier publications have reported high-performance liquid chromatography (HPLC)[5] and high performance thin-layer chromatography (HPTLC)[6] methods for quantification of Cefprozil in human plasma and pharmaceutical dosage form. However, these methods involve arduous sample preparation

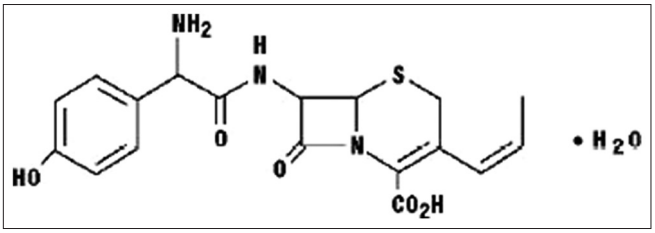

Figure 1: Structure of Cefprozil

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and long chromatographic run times for biological samples. Gowrisankar *et al*., reported UV spectroscopic method, but it requires various solvents and involves many complex reactions.[7] Hence, the objective of this research work was to develop and validate the method for its precision, linearity, and accuracy as per International Conference on Harmonization guidelines (ICH) guidelines. Furthermore, application of this spectrophotometric method to study the stress degradation behavior of Cefprozil was evaluated as per ICH Q1 AR2 guidelines.

Materials and Methods

Instrument and materials

Instrument used were Schimadzu 1800 double beam UV/Visible Spectrophotometer and Schimadzu 1600 analytical balance (Japan). Drug was obtain from Unimark Remedies Limited (Mumbai, Maharashtra, India) as gift sample with 99.9% w/w assay value and was used without further purification. All chemicals and reagents used were of analytical grade.

Preparation of standard stock solution

Standard drug solution of Cefprozil was prepared by dissolving 10 mg Cefprozil in 10 ml distilled water to obtain a stock solution of 1 mg/ml concentration. This was followed by sonication for 15 min and warming to dissolve it. It was finally filtered through Whatmann filter paper #41.

Preparation of calibration curve

Aliquots of 0.1-1 ml portion of stock solutions were transferred to a series of 100-ml volumetric flasks, and volume was made up to mark with distilled water. Solutions were scanned in the range of 200-400 nm against blank. The absorption maxima were found to be at 280 nm against blank. The calibration curve was plotted. The optical characteristics are summarized in Table 1 [Figure 2].

Preparation of sample solution

The proposed method was applied to analyze commercially available Cefprozil tablet. Ten tablets were weighed and powdered. The amount of tablet powder equivalent to 10 mg of Cefprozil was weighed accurately and transfer to 10-ml volumetric flask then 5 ml distilled water was added and kept for 15 min with frequent shaking and volume was made up to the mark with distilled water. The solution was then filtered through Whatmann filter paper #41. This filtrate was diluted suitably with distilled water to get the solution of 10 µg/ml concentration. The absorbance was measured against solution blank. The drug content of the preparation was calculated using standard calibration curve. Amount of drug estimated by this method in Table 2.

Assay of Cefprozil tablets

The proposed method was applied to analyze commercially available Cefprozil tablet (Cefzil®). Twenty tablets were weighed and powdered. The amount of let powder equivalent to 250 mg of Cefprozil was weighed accurately and transferred to 1000-ml volumetric flask. Sufficient quantity of distilled water was added to the volumetric flask, followed by frequent shaking for 15 min to dissolve the content. Finally, the volume was made up using distilled water. The solution was then filtered through Whatmann filter paper #41 and finally diluted to get the solution of concentration 5 µg/ml. The absorbance was measured against a blank solution. The drug content of the solution was calculated using standard calibration

Table 1: Calibration curve of Cefprozil

Concentration in µg/ml	Absorbance
2	0.219
3	0.332
4	0.445
5	0.544
6	0.661
7	0.783
8	0.896
9	0.999
10	1.104

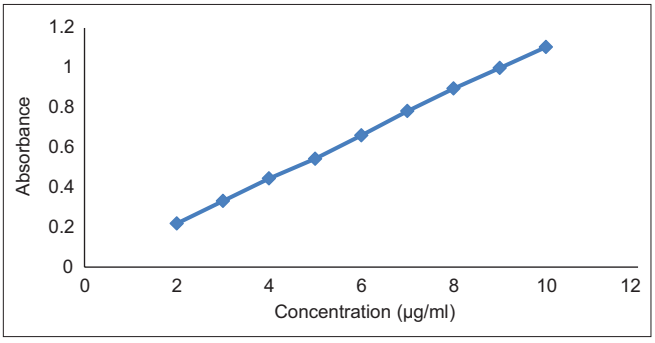

Figure 2: Calibration curve of Cefprozil (2-10 μg/mL)

Table 2: Determination of accuracy by percentage recovery method

Ingredient	Tablet amount[a] (cefzil®) (µg/ml)	Level of addition (%)	Amount added[a] (µg/ml)	Total amount taken from tablet[a] (µg/ml)	Amount recovered (µg/ml)	% Recovery	Average %recovery
Cefprozil	5.00	80	4.00	9.00	8.94	99.33	99.117±1.005
	5.00	100	5.12	10.12	9.92	98.023	
	5.00	120	6.01	11.01	11.00	100	

[a]Amount equivalent to pure drug

curve. Amount of drug estimated by this method is shown in Table 3.

PART I: Method validation

Linearity

Aliquots ranging 0.1-1 ml portion of stock solutions were transferred to series of 100-ml volumetric flasks and volume was made up to the mark with distilled water as shown in Table 1. Solutions were scanned in the range of 200-400 nm against blank. The absorption maxima were found to be at 280 nm against blank. The linearity of measurement was evaluated by analyzing different concentrations of the standard solution of Cefprozil. The calibration curve was obtained by plotting the absorbance versus the concentration as shown in Table 1 and was treated by linear regression analysis.

Accuracy

To ascertain the accuracy of the proposed methods, recovery studies were carried out by the standard addition method at three different levels (80%, 100%, and 120%) of the bulk sample of Cefprozil to the previously analyzed solution of formulation of concentration of 10 μg/ml. Accuracy was determined by the procedure mentioned in assay of Cefprozil [Table 2].

Specificity

Specificity is a procedure to detect quantitatively the analyte in the presence of component that may be expected to be present in the sample matrix. Commonly used excipients in tablet preparation were spiked in a pre-weighed quantity of drug, followed by measurement of absorbance and calculation to determine quantity of drugs.

Precision

The precision of the method was achieved by replicate (*n*=6) analysis of tablet preparations. To study intra-day variation, standard solutions containing 5 μg/ml was prepared and analyzed. This procedure was repeated after regular interval for 6 times on the same day. To study inter-day variation, standard solutions containing 5 μg/ml was prepared and analyzed daily for 6 days. The intra-day and inter-day variations were calculated in terms of percentage relative standard deviation, and the results are given in Table 4.

PART II: Forced degradation studies

The ICH guideline entitled stability testing of new drug substances and products requires that stress testing be carried out to elucidate the inherent stability characteristics of the active substance. Stability studies of Cefprozil were carried out under extreme stress conditions like acidic, alkaline, hydrolytic, thermolytic, oxidation, photolytic, and UV exposure as per stability indicating assay methods (SIAM).

Table 3: Assay of marketed formulation (cefzil®)

Formulation	Label claim (mg/tablet)	Amount found (mg)	Percent label claim
Cefzil® tablet	250	248.85	99.54±0.921

Table 4: Results of precision study

Sample number	Assay of Cefprozil as percentage (%) of labeled amount	
	Analyst-I (intraday precision)	Analyst-II (interday precision)
1	99.72	99.77
2	99.93	99.97
3	99.78	99.71
4	99.90	99.88
5	99.48	99.51
6	99.20	99.25
Mean	99.67	99.68
SD	0.28	0.26

Acid- and base-induced degradation studies

Acid decomposition studies were performed by using 0.1, 0.2, 0.5, and 1 N HCl and alkali-induced decomposition studies were performed by using 0.1 0.2, and 0.5 N NaOH. Appropriate concentration of drug solution was exposed to 0.1 N HCl for 4 h and absorbance was measured at intervals of 1 h. Similar procedure was followed for different concentration of 1 N HCl as 0.2, 0.5 and 0.1, 0.2, and 0.5 N NaOH.

Hydrogen peroxide-induced degradation

To study hydrogen peroxide-induced degradation, initial studies were performed in 3% hydrogen peroxide at room temperature for 4 h. Subsequently, the drug was exposed to 30% hydrogen peroxide at room temperature for a period of 4 h. Appropriate concentration of drug solution was exposed to 3% hydrogen peroxide for 4 h and absorbance was measured at an interval after every 1 h. For the spectroscopic studies, the resultant solutions were scanned between 400 and 200 nm and absorbance was measured.

Photolytic degradation

Photo-degradation studies were performed by directly exposing Cefprozil to sunlight during daytime (60,000-70,000 lux) for 2 days. Then, 10 μg/ml of the solution was scanned in UV spectrophotometer after every 1 h.

Neutral hydrolysis

To study the degradation behavior of drugs under neutral conditions, drug was dissolved in distilled water, the solution was kept for 4 h, and absorbance was measured after every 1 h.

Results and Discussion

PART I: Validation of method

Accuracy and recovery studies

The percentage recovery for Cefprozil by all the methods was found in the range of 99.117±1.005, as shown in Tables 2 and 5.

Linearity

The equation of the calibration curve for Cefprozil obtained was y=0.111x–0.003, the calibration curve was found to be linear in the aforementioned concentrations [the correlation coefficient (r^2) of determination was 0.999] [Figure 2 and Table 5].

Precision

Assay of method precision (intraday precision) was evaluated by carrying out six independent assays of test samples of Cefprozil. The intermediate precision (interday precision) of the method was also evaluated using two different analysts, systems, and different days in the same laboratory. The relative standard deviation (RSD) and assay values obtained by two analysts were 0.28, 99.67 and 0.26, 99.68, respectively [Tables 4 and 5].

PART II: Forced degradation studies

Acid degradation

The acid degradation was performed using 0.1 N HCl. The overlay graph of Cefprozil and acid-degraded Cefprozil is shown in Figure 3. Reduced intensity of the Cefprozil peak at 280 nm indicates acid degradation of Cefprozil. Peak 1 in Figure 3 represents formation of degraded product when compared to the peaks of pure drug. The percentage concentration of degraded drug was calculated. It was found that Cefprozil is degraded within 1 h. The results are shown in Table 6.

Alkali degradation

The alkali degradation was performed using 0.1, 0.2, and 0.5 N NaOH. The overlay graph of Cefprozil and alkali-degraded Cefprozil is shown in Figures 4-6, respectively. It reflects the reduced intensity of peak at 280 nm, the reason for which can be attributed to the formation of degradation products. Inversely, increase in the intensity of peak at 180 nm indicates the formation of degraded product when compared to the peaks of pure drug. The percentage concentration of degraded drug was calculated. It was found that Cefprozil is degraded within 1 h. The results are shown in Table 7.

Neutral degradation

The neutral degradation was performed. The overlay graph of Cefprozil and neutral-degraded Cefprozil is shown in Figure 7. The percentage of degraded drug was calculated. It was found that Cefprozil is degraded within 2 h. The results are shown in Table 8.

Table 5: Validation parameters

Parameter	Result
Absorption maxima (nm)	280
Linearity range (μg/ml)	2-10
Standard regression equation	y=0.111x–0.003
Correlation coefficient (r^2)	0.999
Molar absorptivity	42639.3
A (1%, 1 cm)	1095
Accuracy (% recovery±SD)	99.117
Precision (% CV)	0.28
LOD (μg/ml)	1.00
LOQ (μg/ml)	1.92

LOD – Limit of detection; LOQ – Limit of quantitation

Table 6: Acid degradation of Cefprozil

Name (hr)	Absorbance	Concentration (mg)	Degradation (%)
Analyte at 0	0.984	0.0089	11
Analyte at 1	0.358	0.0032	68
Analyte at 2	0.340	0.00310	68
Analyte at 3	0.340	0.00310	68
Analyte at 4	0.341	0.00315	68

Table 7: Alkali degradation of Cefprozil

Name (hr)	Absorbance	Concentration (mg)	Degradation (%)
Analyte at 0	0.972	0.00887	11.3
Analyte at 1	0.225	0.00205	79.5
Analyte at 2	0.224	0.00204	79.5
Analyte at 3	0.226	0.00206	79.5
Analyte at 4	0.224	0.00204	79.5

Table 8: Neutral degradation of Cefprozil

Name (hr)	Absorbance	Concentration (mg)	Degradation (%)
Analyte at 0	1.012	0.009242	7.56
Analyte at 1	0.969	0.008849	11.50
Analyte at 2	0.160	0.00146	85.4
Analyte at 3	0.154	0.00140	86
Analyte at 4	0.164	0.00146	86

Photolytic degradation

The photolytic degradation was performed using sunlight; the overlay graph of Cefprozil and photolytic-degraded Cefprozil are shown in Figure 8. The percentage of degraded drug was calculated and Cefprozil was found to be degraded in 2 h. The results are shown in Table 9.

Oxidative degradation

Oxidative degradation was performed by using 30% hydrogen peroxide. The percentage of degraded drug was calculated. It was found that Cefprozil is degraded within 1 h. The results are shown in Table 10.

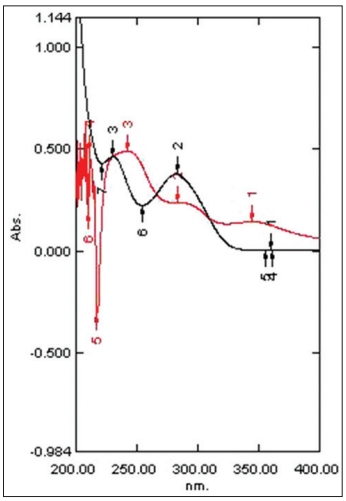

Figure 3: The overlay graph of Cefprozil and acid-degraded (0.1 N HCl) Cefprozil

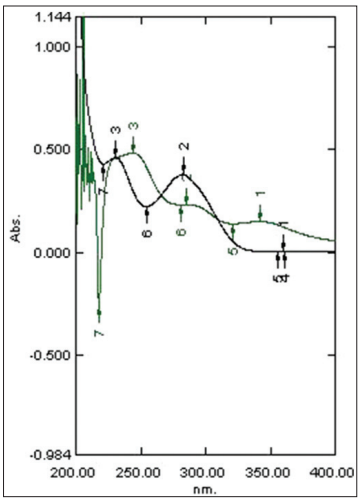

Figure 4: Overlay graph of Cefprozil and alkali-degraded (0.1 N NaOH) Cefprozil

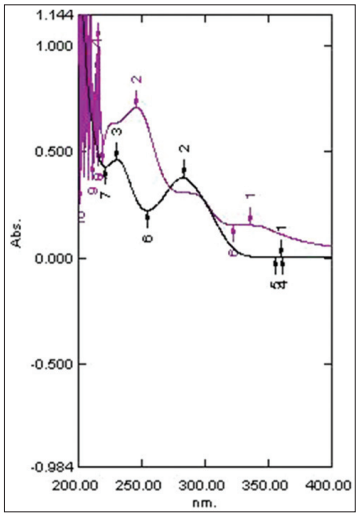

Figure 5: Overlay graph of Cefprozil and alkali-degraded (0.2 N NaOH) Cefprozil

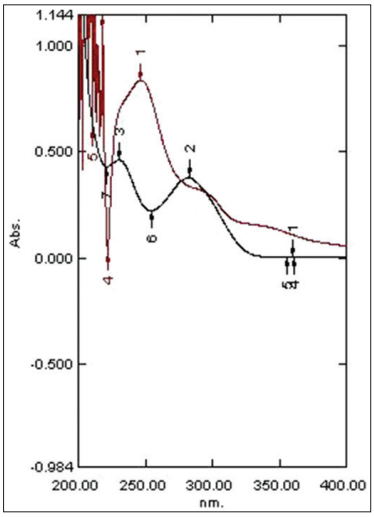

Figure 6: Overlay graph of Cefprozil and alkali-degraded (0.5 N NaOH) Cefprozil

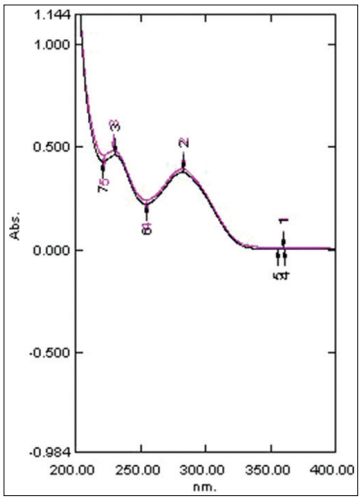

Figure 7: Overlay graph of Cefprozil and neutral-degraded Cefprozil

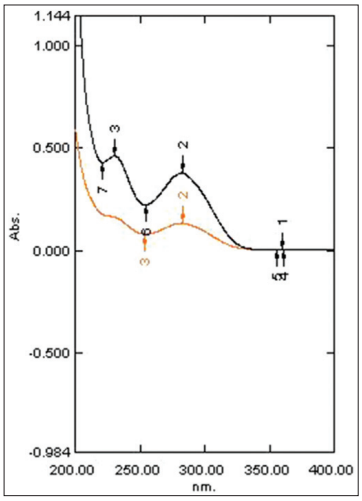

Figure 8: Overlay graph of Cefprozil and photolytic-degraded Cefprozil

Table 9: Photolytic degradation of Cefprozil

Name (hr)	Absorbance	Concentration (mg)	Degradation (%)
Analyte at 0	0.984	0.0089	11
Analyte at 1	0.374	0.0034	66
Analyte at 2	0.359	0.00327	68
Analyte at 3	0.345	0.003150	69
Analyte at 4	0.346	0.00315	69

Table 10: Oxidative degradation of Cefprozil

Name (hr)	Absorbance	Concentration (mg)	Degradation (%)
Analyte at 0	1.012	0.009242	7.56
Analyte at 1	0.147	0.001342	86.58
Analyte at 2	0.154	0.00140	86
Analyte at 3	0.111	0.0010137	90
Analyte at 4	0.054	0.0004	96

Conclusion

A simple, new UV spectroscopic method was developed and validated. It was used to study the stress-degradation studies as per ICH guidelines. Cefprozil was found to be degraded in all types of stress conditions within 1 h and was found to be least stable. The proposed method is accurate, precise, reproducible, and economical and can be successfully used for routine analysis of Cefprozil and evaluation of the stability to quantify Cefprozil and its degradation products in its pharmaceutical dosage form. This method will be highly useful for small-scale industries, where the other high-end instruments are not available.

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